

WHAT IS CLAIMED IS:

1. A method of recombining a set of nucleic acid fragments, the method comprising:

5 hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded nucleic acid templates and a second set of nucleic acids comprises at least one set of nucleic acid fragments;

cleaving one or more unhybridized portions of hybridized nucleic acid fragments; and,

10 elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set of nucleic acid fragments.

2. The method of claim 1, wherein at least one of the cleaving, elongating, or ligating steps is conducted in vivo or in vitro.

3. The at least substantially full-length chimeric nucleic acid sequences made by the method of claim 1.

4. The method of claim 1, comprising providing the first set of nucleic acids to comprise nucleic acids selected from the group consisting of: sense cDNA sequences, antisense cDNA sequences, sense DNA sequences, antisense DNA sequences, sense RNA sequences, and antisense RNA sequences.

5. The method of claim 1, further comprising providing the single-stranded nucleic acid templates, the method comprising:

25 amplifying one or more double-stranded template nucleic acids, wherein each primer of a first of two primer sets comprises a 5' terminal phosphate; and,

degrading one strand of each amplicon with at least one nuclease, wherein the degraded strand comprises the 5' terminal phosphate, thereby providing the single-stranded nucleic acid templates.

0924507-14200

6. The method of claim 5, comprising synthesizing primers of the first primer set with the 5' terminal phosphate.

7. The method of claim 5, comprising phosphorylating a 5' terminal of each member of the first primer set with a kinase prior to the amplifying step.

5 8. The method of claim 5, wherein the at least one nuclease comprises a lambda exonuclease.

9. The method of claim 1, further comprising providing the single-stranded nucleic acid templates, the method comprising:

10 amplifying one or more double-stranded template nucleic acids, wherein each primer of a first of two primer sets comprises one or more 5' terminal phosphorothioates; and,

degrading one strand of each amplicon with at least one nuclease, wherein the degraded strand lacks the one or more 5' terminal phosphorothioates, thereby providing the single-stranded nucleic acid templates.

15 10. The method of claim 9, wherein each member of the first primer set comprises 1, 2, 3, 4, 5, or more 5' terminal phosphorothioates.

11. The method of claim 9, wherein the at least one nuclease comprises a T7 gene 6 exonuclease.

20 12. The method of claim 1, comprising providing one or more vectors to comprise at least one member of the first set of nucleic acids.

13. The method of claim 1, wherein the ligating step comprises contacting the hybridized nucleic acid fragments with at least one nucleic acid ligase.

14. The method of claim 13, wherein the at least one nucleic acid ligase exhibits a gap repair activity.

15. The method of claim 13, wherein the at least one nucleic acid ligase is selected from the group consisting of: a T4 RNA ligase, a T4 DNA ligase, and an E. coli DNA ligase.

16. The method of claim 1, wherein the elongating step comprises
5 contacting the hybridized nucleic acid fragments with at least one polymerase.

17. The method of claim 16, wherein the at least one polymerase comprises a strand non-displacing DNA polymerase.

18. The method of claim 16, wherein the at least one polymerase comprises at least one thermostable polymerase.

10 19. The method of claim 16, wherein the at least one polymerase comprises an intrinsic exonuclease activity.

20 20. The method of claim 16, wherein the at least one polymerase is selected from the group consisting of: a Kornberg DNA polymerase I, a Klenow DNA polymerase I polymerase, a T4 DNA polymerase, a T7 DNA polymerase, a Taq DNA polymerase, a Micrococcal DNA polymerase, an alpha DNA polymerase, an AMV reverse transcriptase, an M-MuLV reverse transcriptase, an E. coli RNA polymerase, an SP6 RNA polymerase, a T3 RNA polymerase, a T7 RNA polymerase, and an RNA polymerase II.

21. The method of claim 1, comprising providing the first and second
20 sets of nucleic acids to comprise substantially homologous sequences.

22. The method of claim 1, wherein the second set of nucleic acids comprises a standardized or a non-standardized set of nucleic acids.

23. The method of claim 1, wherein the second set of nucleic acids comprises a stochastic or a nonstochastic set of the nucleic acid fragments.

25 24. The method of claim 1, wherein the second set of nucleic acids to comprise chimeric nucleic acid sequence fragments.

25. The method of claim 1, wherein the second set of nucleic acids from the group consisting of: cultured microorganisms, uncultured microorganisms, complex biological mixtures, tissues, sera, pooled sera or tissues, multispecies consortia, fossilized or other nonliving biological remains, environmental isolates, soils, groundwaters, waste facilities, and deep-sea environments.

26. The method of claim 1, wherein the first or second set of nucleic acids is synthesized.

27. The method of claim 1, wherein the second set of nucleic acids is derived from the group consisting of: individual cDNA molecules, cloned sets of cDNAs, cDNA libraries, extracted RNAs, natural RNAs, in vitro transcribed RNAs, characterized genomic DNAs, uncharacterized genomic DNAs, cloned genomic DNAs, genomic DNA libraries, enzymatically fragmented DNAs, enzymatically fragmented RNAs, chemically fragmented DNAs, chemically fragmented RNAs, physically fragmented DNAs, and physically fragmented RNAs.

28. The method of claim 1, wherein the single-stranded nucleic acid templates each comprise at least one affinity-label.

29. The method of claim 1, wherein the elongating step is controlled by varying a reaction temperature.

30. The method of claim 1, wherein at least one of the single-stranded nucleic acid templates comprises one or more target nucleic acids that encodes a polypeptide selected from the group consisting of: monooxygenases, cytochrome P450s, glutathione sulfur-transferases (GSTs), homoglutathione sulfur-transferases (HGSTs), P450 monooxygenases, glyphosate oxidases, phosphinothricin acetyl transferases, dichlorophenoxyacetate monooxygenases, acetolactate synthases, 5-enol pyruvylshikimate-3-phosphate synthases, UDP-N-acetylglucosamine enolpyruvyltransferases, glutathione sulfur transferases from maize, homoglutathione sulfur transferases from soybean, glyphosate oxidases from bacteria, phosphinothricin acetyl transferases from bacteria, dichlorophenoxyacetate monooxygenases from

- bacteria, acetolactate synthases from one or more plants, protoporphyrinogen oxidases from one or more plants, protoporphyrinogen oxidases from one or more algae, 5-enolpyruvylshikimate-3-phosphate synthases from one or more plants, 5-enolpyruvylshikimate-3-phosphate synthases from one or more bacteria, UDP-N-acetylglucosamine enolpyruvyltransferases from one or more bacteria, Acetolactate synthases, Acetolactate synthases from *Arabidopsis*, Acetolactate synthases from cotton, Acetolactate synthases from barley, Bt toxins, cry1Aa1, cry1Aa2, cry1Aa3, cry1Aa4, cry1Aa5, cry1Aa6, cry1Ab1, cry1Ab2, cry1Ab3, cry1Ab4, cry1Ab5, cry1Ab6, cry1Ab7, cry1Ab8, cry1Ab9, cry1Ab10, cry1Ac1, cry1Ac2, cry1Ac3, cry1Ac4, cry1Ac5, cry1Ac6, cry1Ac7, cry1Ac8, cry1Ac9, cry1Ac10, cry1Ad1, cry1Ae1, cry1Af1, cry1Ba1, cry1Ba2, cry1Bb1, cry1Bc1, cry1Bd1, cry1Ca1, cry1Ca2, cry1Ca3, cry1Ca4, cry1Ca5, cry1Ca6, cry1Ca7, cry1Cb1, cry1Da1, cry1Db1, cry1Ea1, cry1Ea2, cry1Ea3, cry1Ea4, cry1Eb1, cry1Fa1, cry1Fa2, cry1Fb1, cry1Fb2, cry1Ga1, cry1Ga2, cry1Gb1, cry1Ha1, cry1Hb1, cry1Ia1, cry1Ia2, cry1Ia3, cry1Ia4, cry1Ia5, cry1Ib1, cry1Ic1, cry1Ja1, cry1Jb1, cry1Ka1, cry2Aa1, cry2Aa2, cry2Aa3, cry2Aa4, cry2Ab1, cry2Ab2, cry2Ac1, cry3Aa1, cry3Aa2, cry3Aa3, cry3Aa4, cry3Aa5, cry3Aa6, cry3Ba1, cry3Ba2, cry3Bb1, cry3Bb2, cry3Ca1, cry4Aa1, cry4Aa2, cry4Ba1, cry4Ba2, cry4Ba3, cry4Ba4, cry5Aa1, cry5Ab1, cry5Ac1, cry5Ba1, cry6Aa1, cry6Ba1, cry7Aa1, cry7Ab1, cry7Ab2, cry8Aa1, cry8Ba1, cry8Ca1, cry9Aa1, cry9Aa2, cry9Ba1, cry9Ca1, cry9Da1, cry9Da2, cry9Ea1, cry10Aa1, cry11Aa1, cry11Aa2, cry11Ba1, cry11Bb1, cry11Bb1, cry12Aa1, cry13Aa1, cry14Aa1, cry15Aa1, cry16Aa1, cry17Aa1, cry18Aa1, cry19Aa1, Cry19Ba1, cry20Aa1, cry21Aa1, cry22Aa1, cry24Aa1, cry25Aa1, cry26Aa1, cry28Aa1, cyt1Aa1, cyt1Aa2, cyt1Aa3, cyt1Aa4, cyt1Ab1, cyt1Ba1, cyt2Aa1, cyt2Ba1, cyt2Ba2, cyt2Ba3, cyt2Ba4, cyt2Ba5, cyt2Ba6, cyt2Bb1, 40kDa, cryC35, cryTDK, cryC53, vip1A, vip2A, vip3A(a), vip3A(b), p21med, α -amylase inhibitors, cholesterol oxidases, polyphenol oxidases, insecticidal proteases, vegetative insecticidal proteins, pathways for synthesis of one or more polyketides, cyp 1, cyp 2, cyp 3, peroxidases, chlorperoxidases, iron-sulfur methane monooxygenases, trichothecene-3-O-acetyltransferases, 3-O-Methyltransferases, glutathione S-transferases, epoxides, hydrolases, isomerases, macrolide-O-acetyltransferases, 3-O-acetyltransferases, cis-diol producing monooxygenases for furan, ADP-glucose pyrophosphorylases, ribulose 1,5-bisphosphate carboxylase/oxygenases,

Calvin cycle enzymes, Krebs cycle enzymes, Phosphoenolpyruvate (PEP) carboxylases, Acetyl-CoA carboxylases, homomeric acetyl-CoA carboxylases, heteromeric acetyl-CoA carboxylases, heteromeric acetyl-CoA carboxylases, BCCP subunits, heteromeric acetyl-CoA carboxylases (alpha)-CT subunits, heteromeric acetyl-CoA carboxylase (beta)-CT subunits, acyl carrier proteins (ACP), malonyl-CoA:ACP transacylases, ketoacyl-ACP synthases (KAS), KAS I, KAS II, KAS III, ketoacyl-ACP reductases, 3-hydroxyacyl-ACPs, enoyl-ACP reductases, stearyl-ACP desaturases, acyl-ACP thioesterases, FatA, FatB, glycerol-3-phosphate acyltransferases, 1-acyl-sn-glycerol-3-phosphate acyltransferases, plastidial cytidine-5'-diphosphate-diacylglycerol synthases, plastidial phosphatidylglycero-phosphate synthases, plastidial phosphatidylglycerol-3-phosphate phosphatases, phosphatidylglycerol desaturases, plastidial oleate desaturase (fad6), plastidial linoleate desaturase (fad7/fad8), plastidial phosphatidic acid phosphatase, monogalactosyldiacyl-glycerol synthases, monogalactosyldiacyl-glycerol desaturases, digalactosyldiacyl-glycerol synthases, sulfolipid biosynthesis proteins, long-chain acyl-CoA synthetases, ER glycerol-3-phosphate acyltransferases, ER 1-acyl-sn-glycerol-3-phosphate acyltransferases, ER phosphatidic acid phosphatases, diacylglycerol cholinephosphotransferases, ER oleate desaturases, fad2, ER linoleate desaturases fad3, ER cytidine-5'-diphosphate-diacylglycerol synthases, ER phosphatidylglycero-phosphate synthases, ER phosphatidylglycerol-3-phosphate phosphatases, phosphatidylinositol synthases, diacylglycerol kinases, cholinephosphate cytidyltransferases, phosphatidylcholine transfer proteins, choline kinases, Lipases, phospholipase Cs, phospholipase Ds, phosphatidylserine decarboxylases, phosphatidylinositol-3-kinases, ketoacyl-CoA synthases (KCSs), (beta)-keto-acyl reductases, transcription factors, CER 2, fatty acid isomerases, fatty acid hydroxylases, fatty acid epoxidases, fatty acid acetylenases, methyl transferase related enzymes which alters lipid, cyclopropane fatty acid synthases, meromycolic acid synthases, cyclopropane mycolic acid synthases, diacylglycerol acyltransferases (DGAT), acyl CoA reductases, wax synthases, Cholesterol:Acyl-CoA acyltransferases (ACATs), lecithen:Acyl-CoA Acyltransferases (LCAT), NSMEs, starch synthases, starch synthetases, amylases, alpha amylases, beta amylases, branching enzymes (BEs), BEI, BEIIa, BEIIb, BEIII, debranching enzymes, isoamylases, pullulanases, starch phosphorylases, R genes, Bs2, Cf2, Cf4, Cf9, Hcr2,

- Hcr9, Xa21, Rpl-D, Rpp5, Rpp8, RPM1, RPS2, RPS4, PRF, L6, M, I2, N, Rx, Mi, Dm3, Xa1, Pib, Pto, Pti1, Mlo, Hs1pro-1, LRK10, an agrobacterium vector, Fen, vir A, vir B, vir C, vir D, vir E, vir G, chvE, erythropoietin (EPO), insulin, peptide hormones, human growth hormone, growth factors, cytokines, epithelial Neutrophil Activating Peptide-78,
- 5 GRO α /MGSA, GRO β , GRO γ , MIP-1 α , MIP-1 α , MCP-1, epidermal growth factors, fibroblast growth factors, hepatocyte growth factors, insulin-like growth factors, interferons, interleukins, keratinocyte growth factors, leukemia inhibitory factors, oncostatin M, PD-ECSF, PDGF, pleiotropin, SCF, c-kit ligand, VEGF, G-CSF, IL-1, IL-2, IL-8, FGF, IGF-I, IGF-II, FGF, PDGF, TNF, TGF- α , TGF- β , EGF, KGF, SCF/c-
- 10 Kit, CD40L/CD40, VLA-4/VCAM-1, ICAM-1/LFA-1, hyalurin/CD44, Mos, Ras, Raf, Met, transcriptional activators, transcriptional suppressors, p53, Tat, Fos, Myc, Jun, Myb, Rel, steroid hormone receptors, estrogen receptors, progesterone receptors, testosterone receptors, aldosterone receptors, LDL receptor ligands, corticosterone, Rnases, Onconase, EDN, Alpha-1 antitrypsins, Angiostats, Antihemolytic factors, Apolipoproteins,
- 15 Apoproteins, Atrial natriuretic factors, Atrial natriuretic polypeptides, Atrial peptides, C-X-C chemokines, T39765, NAP-2, ENA-78, Gro-a, Gro-b, Gro-c, IP-10, GCP-2, NAP-4, SDF-1, PF4, MIG, Calcitonins, CC chemokines, Monocyte chemoattractant protein-1, Monocyte chemoattractant protein-2, Monocyte chemoattractant protein-3, Monocyte inflammatory protein-1 alpha, Monocyte inflammatory protein-1 beta, RANTES, I309,
- 20 R83915, R91733, HCC1, T58847, D31065, T64262), CD40 ligand, Collagen, Colony stimulating factor (CSF), Complement factor 5a, Complement inhibitors, Complement receptor 1, Factor IX, Factor VII, Factor VIII, Factor X, Fibrinogen, Fibronectin, Glucocerebrosidases, Gonadotropins, Hedgehog proteins, Hemoglobins, Hirudins, Human serum albumins, Lactoferrins, Luciferases, Neurturins, Neutrophil inhibitory
- 25 factors (NIFs), Osteogenic proteins, Parathyroid hormones, Protein A, Protein G, Relaxins, Renins, Salmon calcitonins, Salmon growth hormones, Soluble complement receptor I, Soluble I-CAM 1, Soluble interleukin receptors, Soluble TNF receptors, Somatomedins, Somatostatins, Somatotropins, Streptokinases, Superantigens, SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, toxic shock syndrome toxin (TSST-1), Exfoliating
- 30 toxins A and B, Pyrogenic exotoxins A, B, and C, and M. arthritides mitogen, Superoxide dismutase, Thymosin alpha 1, Tissue plasminogen activator, Tumor necrosis factor beta

(TNF beta), Tumor necrosis factor receptor (TNFR), Tumor necrosis factor-alpha (TNF alpha) and Urokinases.

31. The method of claim 1, wherein the at least one set of nucleic acid fragments is derived by fragmentation or synthesis from one or more target nucleic acid
5 which encodes a polypeptide selected from the group consisting of: monooxygenases, cytochrome P450s, glutathione sulfur-transferases (GSTs), homoglutathione sulfur-transferases (HGSTs), P450 monooxygenases, glyphosate oxidases, phosphinothricin acetyl transferases, dichlorophenoxyacetate monooxygenases, acetolactate synthases, 5-enol pyruvylshikimate-3-phosphate synthases, UDP-N-acetylglucosamine
10 enolpyruvyltransferases, glutathione sulfur transferases from maize, homoglutathione sulfur transferases from soybean, glyphosate oxidases from bacteria, phosphinothricin acetyl transferases from bacteria, dichlorophenoxyacetate monooxygenases from bacteria, acetolactate synthases from one or more plants, protoporphyrinogen oxidases from one or more plants, protoporphyrinogen oxidases from one or more algae, 5-enolpyruvylshikimate-3-phosphate synthases from one or more plants, 5-enolpyruvylshikimate-3-phosphate synthases from one or more bacteria, UDP-N-acetylglucosamine enolpyruvyltransferases from one or more bacteria, Acetolactate synthases, Acetolactate synthases from *Arabidopsis*, Acetolactate synthases from cotton, Acetolactate synthases from barley, Bt toxins, cry1Aa1, cry1Aa2, cry1Aa3, cry1Aa4,
20 cry1Aa5, cry1Aa6, cry1Ab1, cry1Ab2, cry1Ab3, cry1Ab4, cry1Ab5, cry1Ab6, cry1Ab7, cry1Ab8, cry1Ab9, cry1Ab10, cry1Ac1, cry1Ac2, cry1Ac3, cry1Ac4, cry1Ac5, cry1Ac6, cry1Ac7, cry1Ac8, cry1Ac9, cry1Ac10, cry1Ad1, cry1Ae1, cry1Af1, cry1Ba1, cry1Ba2, cry1Bb1, cry1Bc1, cry1Bd1, cry1Ca1, cry1Ca2, cry1Ca3, cry1Ca4, cry1Ca5, cry1Ca6, cry1Ca7, cry1Cb1, cry1Da1, cry1Db1, cry1Ea1, cry1Ea2, cry1Ea3, cry1Ea4, cry1Eb1, cry1Fa1, cry1Fa2, cry1Fb1, cry1Fb2, cry1Ga1, cry1Ga2, cry1Gb1, cry1Ha1, cry1Hb1,
25 cry1Ia1, cry1Ia2, cry1Ia3, cry1Ia4, cry1Ia5, cry1Ib1, cry1Ic1, cry1Ja1, cry1Jb1, cry1Ka1, cry2Aa1, cry2Aa2, cry2Aa3, cry2Aa4, cry2Ab1, cry2Ab2, cry2Ac1, cry3Aa1, cry3Aa2, cry3Aa3, cry3Aa4, cry3Aa5, cry3Aa6, cry3Ba1, cry3Ba2, cry3Bb1, cry3Bb2, cry3Ca1, cry4Aa1, cry4Aa2, cry4Ba1, cry4Ba2, cry4Ba3, cry4Ba4, cry5Aa1, cry5Ab1, cry5Ac1, cry5Ba1, cry6Aa1, cry6Ba1, cry7Aa1, cry7Ab1, cry7Ab2, cry8Aa1, cry8Ba1, cry8Ca1,
30 cry9Aa1, cry9Aa2, cry9Ba1, cry9Ca1, cry9Da1, cry9Da2, cry9Ea1, cry10Aa1,

- cry11Aa1, cry11Aa2, cry11Ba1, cry11Bb1, cry11Bb1, cry12Aa1, cry13Aa1, cry14Aa1, cry15Aa1, cry16Aa1, cry17Aa1, cry18Aa1, cry19Aa1, Cry19Ba1, cry20Aa1, cry21Aa1, cry22Aa1, cry24Aa1, cry25Aa1, cry26Aa1, cry28Aa1, cyt1Aa1, cyt1Aa2, cyt1Aa3, cyt1Aa4, cyt1Ab1, cyt1Ba1, cyt2Aa1, cyt2Ba1, cyt2Ba2, cyt2Ba3, cyt2Ba4, cyt2Ba5, 5 cyt2Ba6, cyt2Bb1, 40kDa, cryC35, cryTDK, cryC53, vip1A, vip2A, vip3A(a), vip3A(b), p21med, α -amylase inhibitors, cholesterol oxidases, polyphenol oxidases, insecticidal proteases, vegetative insecticidal proteins, pathways for synthesis of one or more polyketides, cyp 1, cyp 2, cyp 3, peroxidases, chlorperoxidases, iron-sulfur methane monoxygenases, trichothecene-3-O-acetyltransferases, 3-O-Methyltransferases, 10 glutathione S-transferases, epoxides, hydrolases, isomerases, macrolide-O-acetyltransferases, 3-O-acetyltransferases, cis-diol producing monooxygenases for furan, ADP-glucose pyrophosphorylases, ribulose 1,5-bisphosphate carboxylase/oxygenases, Calvin cycle enzymes, Krebs cycle enzymes, Phosphoenolpyruvate (PEP) carboxylases, Acetyl-CoA carboxylases, homomeric acetyl-CoA carboxylases, heteromeric acetyl-CoA carboxylases, heteromeric acetyl-CoA carboxylases, BCCP subunits, heteromeric acetyl-CoA carboxylases (alpha)-CT subunits, heteromeric acetyl-CoA carboxylase (beta)-CT subunits, acyl carrier proteins (ACP), malonyl-CoA:ACP transacylases, 15 ketoacyl-ACP synthases (KAS), KAS I, KAS II, KAS III, ketoacyl-ACP reductases, 3-hydroxyacyl-ACPs, enoyl-ACP reductases, stearoyl-ACP desaturases, acyl-ACP 20 thioesterases, FatA, FatB, glycerol-3-phosphate acyltransferases, 1-acyl-sn-glycerol-3-phosphate acyltransferases, plastidial cytidine-5'-diphosphate-diacylglycerol synthases, plastidial phosphatidylglycero-phosphate synthases, plastidial phosphatidylglycerol-3-phosphate phosphatases, phosphatidylglycerol desaturases, plastidial oleate desaturase (fad6), plastidial linoleate desaturase (fad7/fad8), plastidial phosphatidic acid 25 phosphatase, monogalactosyldiacyl-glycerol synthases, monogalactosyldiacyl-glycerol desaturases, digalactosyldiacyl-glycerol synthases, sulfolipid biosynthesis proteins, long-chain acyl-CoA synthetases, ER glycerol-3-phosphate acyltransferases, ER 1-acyl-sn-glycerol-3-phosphate acyltransferases, ER phosphatidic acid phosphatases, diacylglycerol cholinephosphotransferases, ER oleate desaturases, fad2, ER linoleate desaturases fad3, 30 ER cytidine-5'-diphosphate-diacylglycerol synthases, ER phosphatidylglycero-phosphate synthases, ER phosphatidylglycerol-3-phosphate phosphatases, phosphatidylinositol

synthases, diacylglycerol kinases, cholinephosphate cytidyltransferases,
phosphatidylcholine transfer proteins, choline kinases, Lipases, phospholipase Cs,
phospholipase Ds, phosphatidylserine decarboxylases, phosphatidylinositol-3-kinases,
ketoacyl-CoA synthases (KCSs), (beta)-keto-acyl reductases, transcription factors, CER
5 2, fatty acid isomerases, fatty acid hydroxylases, fatty acid epoxidases, fatty acid
acetylenases, methyl transferase related enzymes which alters lipid, cyclopropane fatty
acid synthases, meromycolic acid synthases, cyclopropane mycolic acid synthases,
diacylglycerol acyltransferases (DGAT), acyl C0-A reductases, wax synthases,
Cholesterol:Acyl-CoA acyltransferases (ACATs), lecithen:Acyl-CoA Acyltransferases
10 (LCAT), NSMEs, starch synthases, starch synthetases, amylases, alpha amylases, beta
amylases, branching enzymes (BEs), BEI, BEIIa, BEIIb, BEIII, debranching enzymes,
isoamylases, pullulanases, starch phosphorylases, R genes, Bs2, Cf2, Cf4, Cf9, Hcr2,
Hcr9, Xa21, Rp1-D, Rpp5, Rpp8, RPM1, RPS2, RPS4, PRF, L6, M, I2, N, Rx, Mi, Dm3,
Xa1, Pib, Pto, Pti1, Mlo, Hs1pro-1, LRK10, an agrobacterium vector, Fen, vir A, vir B,
15 vir C, vir D, vir E, vir G and chvE, erythropoietin (EPO), insulin, peptide hormones,
human growth hormone, growth factors, cytokines, epithelial Neutrophil Activating
Peptide-78, GRO α /MGSA, GRO α , GRO β , MIP-1 α , MIP-1 β , MCP-1, epidermal growth
factors, fibroblast growth factors, hepatocyte growth factors, insulin-like growth factors,
interferons, interleukins, keratinocyte growth factors, leukemia inhibitory factors,
20 oncostatin M, PD-ECSF, PDGF, pleiotropin, SCF, c-kit ligand, VEGEF, G-CSF, IL-1,
IL-2, IL-8, FGF, IGF-I, IGF-II, FGF, PDGF, TNF, TGF- α , TGF- β , EGF, KGF, SCF/c-
Kit, CD40L/CD40, VLA-4/VCAM-1, ICAM-1/LFA-1, hyalurin/CD44, Mos, Ras, Raf,
Met, transcriptional activators, transcriptional suppressors, p53, Tat, Fos, Myc, Jun, Myb,
Rel, steroid hormone receptors, estrogen receptors, progesterone receptors, testosterone
25 receptors, aldosterone receptors, LDL receptor ligands, corticosterone, Rnases, Onconase,
EDN, Alpha-1 antitrypsins, Angiostatins, Antihemolytic factors, Apolipoproteins,
Apoproteins, Atrial natriuretic factors, Atrial natriuretic polypeptides, Atrial peptides, C-
X-C chemokines, T39765, NAP-2, ENA-78, Gro-a, Gro-b, Gro-c, IP-10, GCP-2, NAP-4,
SDF-1, PF4, MIG, Calcitonins, CC chemokines, Monocyte chemoattractant protein-1,
30 Monocyte chemoattractant protein-2, Monocyte chemoattractant protein-3, Monocyte
inflammatory protein-1 alpha, Monocyte inflammatory protein-1 beta, RANTES, I309,

R83915, R91733, HCC1, T58847, D31065, T64262), CD40 ligand, Collagen, Colony stimulating factor (CSF), Complement factor 5a, Complement inhibitors, Complement receptor 1, Factor IX, Factor VII, Factor VIII, Factor X, Fibrinogen, Fibronectin, Glucocerebrosidases, Gonadotropins, Hedgehog proteins, Hemoglobins, Hirudins, Human serum albumins, Lactoferrins, Luciferases, Neurturins, Neutrophil inhibitory factors (NIFs), Osteogenic proteins, Parathyroid hormones, Protein A, Protein G, Relaxins, Renins, Salmon calcitonins, Salmon growth hormones, Soluble complement receptor I, Soluble I-CAM 1, Soluble interleukin receptors, Soluble TNF receptors, Somatomedins, Somatostatins, Somatotropins, Streptokinases, Superantigens, SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, toxic shock syndrome toxin (TSST-1), Exfoliating toxins A and B, Pyrogenic exotoxins A, B, and C, and M. arthritides mitogen, Superoxide dismutase, Thymosin alpha 1, Tissue plasminogen activator, Tumor necrosis factor beta (TNF beta), Tumor necrosis factor receptor (TNFR), Tumor necrosis factor-alpha (TNF alpha) and Urokinases.

32. The method of claim 1, further comprising expressing the at least substantially full-length chimeric nucleic acid sequences to provide at least one expression product.

33. The at least one expression product made by the method of claim 32.

34. The method of claim 32, further comprising selecting or screening the at least one expression product for at least one desired trait or property.

35. The method of claim 1, further comprising introducing one or more of the at least substantially full-length chimeric nucleic acid sequences into at least one cell.

36. The at least one cell made by the method of claim 35.

37. The method of claim 35, further comprising expressing the one or more introduced at least substantially full-length chimeric nucleic acid sequences to provide at least one expression product to the at least one cell.

38. The method of claim 37, further comprising selecting or screening the at least one cell for one or more desired traits or properties using at least one plate-based or at least one filter-based assay.

39. The method of claim 1, comprising cleaving the unhybridized
5 portions of the hybridized nucleic acid fragments by nuclease cleavage or by chemical cleavage.

40. The method of claim 1, further comprising separating hybridized nucleic acids from unhybridized nucleic acids by at least one separation technique before or after performing the cleaving step.

10 41. The method of claim 1, further comprising separating hybridized nucleic acids from unhybridized nucleic acids by at least one separation technique before or after performing the cleaving step.

0924507-132200
15 42. The method of claim 1, the method further comprising:
denaturing the at least substantially full-length chimeric nucleic acid sequences
and the single-stranded nucleic acid templates;
separating the at least substantially full-length chimeric nucleic acid sequences
from the single-stranded nucleic acid templates by at least one separation technique; and,
fragmenting the separated at least substantially full-length chimeric nucleic acid
sequences by nuclease digestion or physical fragmentation to provide chimeric nucleic
20 acid sequence fragments.

43. The method of claims 40 or 42, comprising providing the at least one separation technique to comprise a technique selected from the group consisting of: an affinity-based separation, a centrifugation, a fluorescence-based separation, a magnetic field-based separation, an electrophoretic separation, a microfluidic molecular separation,
25 and a chromatographic separation.

44. A method of isolating nucleic acid fragments from a set of nucleic acid fragments, the method comprising:

hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded nucleic acid templates and a second set of nucleic acids comprises at least one set of nucleic acid fragments;

- 5 separating the hybridized nucleic acids from unhybridized nucleic acids by at least one first separation technique; and,
denaturing the separated hybridized nucleic acids to yield the single-stranded nucleic acid templates and isolated nucleic acid fragments.

45. The method of claim 44, wherein the first set of nucleic acids comprises nucleic acids selected from the group consisting of: sense cDNA sequences, antisense cDNA sequences, sense DNA sequences, antisense DNA sequences, sense RNA sequences, and antisense RNA sequences.

46. The method of claim 44, wherein the first and second sets of nucleic acids comprise substantially homologous sequences.

47. The method of claim 44, wherein the second set of nucleic acids comprises a standardized or a non-standardized set of nucleic acids.

48. The method of claim 44, wherein the second set of nucleic acids to comprises chimeric nucleic acid sequence fragments.

49. The method of claim 44, wherein the second set of nucleic acids is derived from the group consisting of: cultured microorganisms, uncultured microorganisms, complex biological mixtures, tissues, sera, pooled sera or tissues, multispecies consortia, fossilized or other nonliving biological remains, environmental isolates, soils, groundwaters, waste facilities, and deep-sea environments.

50. The method of claim 44, wherein the second set of nucleic acids is synthesized.

51. The method of claim 44, wherein the second set of nucleic acids is derived from the group consisting of: individual cDNA molecules, cloned sets of cDNAs, cDNA libraries, extracted RNAs, natural RNAs, in vitro transcribed RNAs, characterized

genomic DNAs, uncharacterized genomic DNAs, cloned genomic DNAs, genomic DNA libraries, enzymatically fragmented DNAs, enzymatically fragmented RNAs, chemically fragmented DNAs, chemically fragmented RNAs, physically fragmented DNAs, and physically fragmented RNAs.

5 **52.** The method of claim 44, wherein the single-stranded nucleic acid templates each comprise at least one affinity-label.

53. The method of claim 44, comprising performing each step sequentially in a single reaction vessel.

10 **54.** The method of claim 44, comprising performing at least one step in at least one reaction vessel separate from other steps.

55. The method of claim 44, further comprising separating the isolated nucleic acid fragments from the single-stranded nucleic acid templates by at least one second separation technique following the denaturing step.

15 **56.** The method of claim 55, wherein the single-stranded nucleic acid templates comprise sense single-stranded nucleic acid templates and wherein the at least one set of nucleic acid fragments comprise at least one set of antisense nucleic acid fragments that correspond to the sense single-stranded nucleic acid templates thereby providing isolated antisense nucleic acid fragments.

20 **57.** The method of claim 55, wherein the single-stranded nucleic acid templates comprise antisense single-stranded nucleic acid templates and the at least one set of nucleic acid fragments which comprise at least one set of sense nucleic acid fragments that correspond to the antisense single-stranded nucleic acid templates thereby providing isolated sense nucleic acid fragments.

25 **58.** The method of claims 44 or 55, wherein the at least one first or the at least one second separation technique to comprise a technique selected from the group consisting of: an affinity-based separation, a centrifugation, a fluorescence-based separation, a magnetic field-based separation, an electrophoretic separation, a

09721507 412200

microfluidic molecular separation, a magnetic separation, and a chromatographic separation.

5 **59.** The method of claim 44, comprising cleaving unhybridized portions of the hybridized nucleic acid fragments by nuclease cleavage before or after the separating step.

60. The method of claim 59, further comprising elongating, ligating, or both; sequence gaps between hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates.

10 **61.** The method of claim 60, further comprising amplifying the at least substantially full-length chimeric nucleic acid sequences.

62. The method of claim 61, further comprising selecting at least one amplified at least substantially full-length chimeric nucleic acid sequence for a desired trait or property of an encoded expression product.

15 **63.** The method of claim 61, further comprising fragmenting the amplified at least substantially full-length chimeric nucleic acid sequences by nuclease digestion or physical fragmentation to provide chimeric nucleic acid sequence fragments.

64. The method of claims 42, 55, or 63, further comprising providing a population of recombined nucleic acids, the method comprising:

20 hybridizing the isolated nucleic acid fragments or the chimeric nucleic acid sequence fragments; and,

 elongating or ligating the hybridized isolated nucleic acid fragments or the hybridized chimeric nucleic acid sequence fragments, thereby providing a population of recombined nucleic acids.

25 **65.** The method of claim 64, wherein the isolated nucleic acid fragments comprise isolated sense and antisense nucleic acid fragments, and wherein the isolated sense nucleic acid fragments correspond to the isolated antisense nucleic acid fragments,

and the method further comprising hybridizing the isolated sense and antisense nucleic acid fragments.

66. The population of recombined nucleic acids made by the method of claim 64.

5 67. The method of claim 64, further comprising introducing one or more members of the population of recombined nucleic acids into at least one cell.

68. The method of claim 67, further comprising expressing the one or more introduced members of the population of recombined nucleic acids to provide at least one expression product to the at least one cell.

10 69. The at least one cell made by the method of claim 67.

70. The method of claim 64, further comprising expressing the population of recombined nucleic acids to provide at least one expression product.

71. The method of claim 70, comprising expressing the population of recombined nucleic acids *in vitro*.

15 72. The method of claim 70, further comprising selecting the at least one expression product for a desired trait or property.

73. The at least one expression product made by the method of claim 70.

20 74. The method of claim 64, the method further comprising:
denaturing the population of recombined nucleic acids;
rehybridizing the denatured population of recombined nucleic acids;
extending the rehybridized population of recombined nucleic acids to provide a
population of further recombined nucleic acids; and, optionally,
repeating the second denaturing, rehybridizing, and extending steps at least once.

25 75. A method of generating chimeric nucleic acids, the method comprising:

hybridizing a first plurality of first parental single-stranded nucleic acids and a second plurality of second parental single-stranded nucleic acids, wherein the hybridized first and second parental single-stranded nucleic acids comprise at least one nonhybridized region of sequence diversity;

5 nicking at least one strand in the at least one nonhybridized region of sequence diversity;

 cleaving the at least one nicked strand in the at least one nonhybridized region of sequence diversity to provide at least one sequence gap between hybridized regions; and,

 elongating, ligating, or both, the at least one sequence gap between the hybridized
10 regions to generate chimeric progeny nucleic acids.

76. The method of claim 75, wherein at least one of the elongating and ligating steps is conducted in vivo.

77. The method of claim 75, wherein at least one of the elongating and ligating steps is conducted in vitro

15 **78.** The method of claim 75, wherein after the ligation step, the hybridized first and second parental single-stranded nucleic acids are transformed into a host.

79. The method of claim 78, wherein the ligated hybridized first and second parental single-stranded nucleic acids comprise at least one nonhybridized region
20 of sequence diversity.

80. The method of claim 75, wherein the nicking step comprises nicking only one strand in the at least one nonhybridized region of sequence diversity.

81. The method of claim 75, further comprising repeating the hybridizing, nicking, cleaving, and elongating steps at least once.

25 **82.** The method of claim 75, wherein the first or second parental single-stranded nucleic acids encode one or more substantially full-length proteins.

83. The method of claim 75, comprising providing the first or second parental single-stranded nucleic acids by performing one or more cycles of an asymmetric polymerase chain reaction.

84. The method of claim 75, comprising providing the first or second parental single-stranded nucleic acids by degrading specific single strands in double-stranded parental sequences with at least one nuclease.

85. The method of claim 84, wherein the at least one nuclease comprises a lambda Exonuclease.

86. The method of claim 75, comprising synthesizing the first or second parental single-stranded nucleic acids.

87. The method of claim 86, further comprising randomly or nonrandomly incorporating dUTP into the first or second parental single-stranded nucleic acids during synthesis.

88. The method of claim 87, the nicking step comprising nicking the at least one strand in the at least one nonhybridized region of sequence diversity at one or more sites of dUTP incorporation with at least one glycosylase and at least one endonuclease.

89. The method of claim 88, wherein the at least one glycosylase comprises a Uracil N-Glycosylase.

90. The method of claim 88, wherein the at least one endonuclease comprises an Endonuclease IV.

91. The method of claim 75, wherein the hybridizing step is performed at a temperature of about 25°C or less.

92. The method of claim 75, the nicking step comprising nicking the at least one strand in the at least one nonhybridized region of sequence diversity with at least one nuclease.

93. The method of claim 92, further comprising controlling a nicking frequency by varying an amount of the at least one nuclease.

94. The method of claim 92, wherein the at least one nuclease comprises a Mung bean nuclease or a nickase.

5 **95.** The method of claim 75, the cleaving step comprising cleaving the at least one nicked strand in the at least one nonhybridized region of sequence diversity with at least one nuclease.

96. The method of claim 95, wherein the at least one nuclease comprises an Exonuclease VII.

10 **97.** The method of claim 75, comprising elongating the at least one sequence gap between the hybridized regions with at least one polymerase.

98. The method of claim 97, wherein the at least one polymerase lacks a strand displacement activity.

15 **99.** The method of claim 97, wherein the at least one polymerase is selected from the group consisting of: a Kornberg DNA polymerase I, a Klenow DNA polymerase I polymerase, a T4 DNA polymerase, a T7 DNA polymerase, a Taq DNA polymerase, a Micrococcal DNA polymerase, an alpha DNA polymerase, an AMV reverse transcriptase, an M-MuLV reverse transcriptase, an E. coli RNA polymerase, an SP6 RNA polymerase, a T3 RNA polymerase, a T7 RNA polymerase, and an RNA
20 polymerase II.

100. The method of claim 75, comprising ligating the at least one sequence gap between the hybridized regions with at least one ligase.

25 **101.** The method of claim 100, wherein the at least one ligase is selected from the group consisting of: a T4 RNA ligase, a T4 DNA ligase, and an E. coli DNA ligase.

102. The method of claim 75, further comprising amplifying the chimeric progeny nucleic acids.

103. The chimeric progeny nucleic acids made by the method of claim 75.

104. A vector comprising one or more of the chimeric progeny nucleic acids made by the method of claim 75.

105. The method of claim 75, further comprising expressing the chimeric progeny nucleic acids to provide at least one expression product.

106. The method of claim 105, further comprising selecting or screening the at least one expression product for one or more desired traits or properties.

107. The at least one expression product made by the method of claim 105.

108. The method of claim 75, further comprising introducing one or more of the chimeric progeny nucleic acids into at least one cell.

109. The method of claim 108, further comprising expressing the introduced chimeric progeny nucleic acids to provide at least one expression product to the at least one cell.

110. The at least one cell made by the method of claim 109.

111. A method of recombining a set of nucleic acid fragments, the method comprising:

hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded sense strand-nucleic acid templates and a second set of nucleic acids consists essentially of single-stranded antisense strand-nucleic acid fragments; and, elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set of nucleic acid fragments.

112. A method of recombining a set of nucleic acid fragments, the method comprising:

hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded antisense strand-nucleic acid templates and a second set of nucleic acids consists essentially of single-stranded sense strand-nucleic acid fragments; and,

10 elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set of nucleic acid fragments.

113. A method of recombining a set of nucleic acid fragments, the method comprising:

hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded nucleic acid templates and a second set of nucleic acids comprises at least one set of nucleic acid fragments; and,

15 elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments by incubating the hybridized nucleic acid fragments with a polymerase and/or a ligase at a temperature of about 45°C or less, to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates,

20 thereby recombining the set of nucleic acid fragments.

114. The method of claim 113, wherein the hybridized nucleic acid fragments are incubated with a polymerase and/or a ligase at a temperature of about 37°C or less.

25 115. The method of claim 113, wherein the hybridized nucleic acid fragments are incubated with a polymerase and/or a ligase at a temperature of about 25°C or less.

116. A method of recombining a set of nucleic acid fragments, the method comprising:

providing a set of at least partially double-stranded nucleic acids that encode a polypeptide of interest or portion thereof;

contacting the set of at least partially double-stranded nucleic acids with an exonuclease that selectively degrades one strand of the at least partially double-stranded nucleic acids to provide a set of single-stranded nucleic acid templates;

5 hybridizing the set of single-stranded nucleic acid templates with a second set of nucleic acids comprising at least one set of nucleic acid fragments; and,

10 elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set of nucleic acid fragments.

117. The method of claim 116, wherein the exonuclease is selected from the group consisting of Exonuclease III, Ba131, Mung bean nuclease, T7 gene 6 exonuclease, and lambda exonuclease.

15 **118.** The method of claim 116, wherein the nucleic acid fragments are single stranded.

119. A method of recombining a set of nucleic acid fragments, the method comprising:

20 hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded nucleic acid templates and a second set of nucleic acids comprises at least one set of nucleic acid fragments;

elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates;

25 introducing one or more of the at least substantially full-length chimeric nucleic acid sequences into at least one cell;

expressing the one or more introduced at least substantially full-length chimeric nucleic acid sequences to provide at least one expression product to the at least one cell; and,

selecting or screening the at least one cell for one or more desired traits or properties using at least one plate-based or at least one filter-based assay.

5 **120.** A method of combinatorially assembling nucleic acids, the method comprising: hybridizing at least two sets of nucleic acids, wherein a first of the at least to sets of nucleic acids comprises single-stranded nucleic acid templates and a second set of the at least two sets of nucleic acids comprises at least one set of nucleic acid fragments, which fragments hybridize to a plurality of subsequences on at least one member of the first set of nucleic acids, wherein hybridization of the first and second set of nucleic acids directs combinatorial assembly of a third set nucleic acids.

10 **121.** The method of claim 120, wherein at least 5 members of the second set of nucleic acids hybridize to one member of the first set of nucleic acids.

122. The method of claim 120, wherein the method further comprises transducing the first and second set of nucleic acids into one or more cells in hybridized form, whereby the cells produce the third set of nucleic acids.

15 **123.** The method of claim 120, wherein the first and second set of nucleic acids are transduced into the cell following treatment with one or more of: a polymerase, a ligase and an exonuclease.

20 **124.** The method of claim 120, wherein the first and second set of nucleic acids are transduced into the cell without treatment by one or more of: a polymerase, a ligase and an exonuclease.

125. The method of claim 120, wherein the first or second set of nucleic acids are homologous.

25 **126.** The method of claim 120, wherein the method further comprises one or more of: digesting the hybridized first and second sets of nucleic acids with one or more nuclease, ligating one or more members of the first or second set of nucleic acids, and extending the first or second set of nucleic acids with a polymerase.

127. The method of claim 120, wherein the hybridized first and second set of nucleic acids provide one or more overlapping sets of nucleic acids.

128. The method of claim 120, further comprising selecting or screening one or more members of the third set of nucleic acids for one or more traits or properties of encoded expression products.

129. The method of claim 120, wherein the trait or property is an enzymatic activity or property.

130. The method of claim 120, wherein the trait or property is screened at a temperature of less than about 20°C or greater than about 50°C, or wherein the trait or property is screened at a pressure of less than about 0.2 atmospheres, or a pressure of greater than about 2 atmospheres, or a pH less than about 5.5, or a pH of greater than about 8.5.

131. The method of claim 120, wherein one or more members of the third set of nucleic acids are selected or screened for an effect on one or more of: immunogenicity, allergenicity, or hypersensitivity.

132. The method of claim 120, wherein one or more members of the third set of nucleic acids are selected or screened in an non-aqueous or a semi-aqueous system.

133. The method of claim 132, wherein one or more cells comprise the one or more members of the third set of nucleic acids.

134. The method of claim 132, wherein the non-aqueous or the semi-aqueous system comprise crude oil or distillation fractions derived therefrom.

135. The method of claim 134, wherein the one or more members of the third set of nucleic acids are screened or selected for an appearance or a disappearance of organic or inorganic sulfur.

136. The method of claim 134, wherein the one or more members of the third set of nucleic acids are screened or selected for a rate or an extent of substrate desulfurization.

137. The method of claim 120, wherein the combinatorial assembly occurs in vitro or in vivo.

138. The method of claim 120, wherein the combinatorial assembly comprises at least one nucleic acid ligase.

139. The method of claim 120, wherein the combinatorial assembly comprises incubation of the first and second nucleic acid sets with one or more engineered or mutant enzyme.

140. The method of claim 138, wherein the at least one nucleic acid ligase exhibits a gap repair activity.

141. The method of claim 138, wherein the at least one nucleic acid ligase is selected from the group consisting of: a T4 RNA ligase, a T4 DNA ligase, and an E. coli DNA ligase.

142. The method of claim 120, wherein the combinatorial assembly comprises at least one polymerase.

143. The method of claim 142, wherein the at least one polymerase comprises a strand non-displacing DNA polymerase.

144. The method of claim 142, wherein the at least one polymerase comprises at least one thermostable polymerase.

145. The method of claim 142, wherein the at least one polymerase comprises an intrinsic exonuclease activity.

146. The method of claim 142, wherein the at least one polymerase is selected from the group consisting of: a Kornberg DNA polymerase I, a Klenow DNA

polymerase I polymerase, a T4 DNA polymerase, a T7 DNA polymerase, a Taq DNA polymerase, a Micrococcal DNA polymerase, an alpha DNA polymerase, an AMV reverse transcriptase, an M-MuLV reverse transcriptase, an E. coli RNA polymerase, an SP6 RNA polymerase, a T3 RNA polymerase, a T7 RNA polymerase, and an RNA polymerase II.

147. The method of claim 120, wherein the combinatorial assembly comprises at least one nuclease.

148. The method of claim 147, wherein the at least one nuclease comprises at least one exonuclease.

149. The method of claim 147, wherein the at least one nuclease comprises a thermostable nuclease.

150. The method of claim 147, wherein the at least one nuclease is selected from the group consisting of: a Bal31 nuclease, an exonuclease III, a Mung bean nuclease, an S1 nuclease, a P1 nuclease, a ribonuclease A, a ribonuclease H, a deoxyribonuclease I, an S7 nuclease, a T7 endonuclease, an exonuclease I, an exonuclease VII, a lambda exonuclease, an N. crassa nuclease, a phosphodiesterase I, and a phosphodiesterase II.

151. The method of claim 120, wherein the combinatorial assembly comprises at least polymerase and at least one ligase.

152. The method of claim 120, wherein the combinatorial assembly comprises at least one ligase and at least one exonuclease.

153. The method of claim 120, wherein the combinatorial assembly comprises at least one nuclease, at least one ligase, and at least one polymerase.

154. The method of claim 120, further comprising moving one or more of the sets of nucleic acids using a robotic arm, a robotic platform, or another computer-controlled electromechanical device prior to the hybridization step.

155. The method of claim 120, further comprising sequencing one or more members of the third set nucleic acids.

156. The method of claim 120, further comprising a logical cataloging step.

5 157. The method of claim 120, further comprising displaying one or more members of the third set nucleic acids or expression products thereof in an array.

158. A method of recombining a set of nucleic acid fragments, the method comprising:

10 hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded sense strand-nucleic acid templates and a second set of nucleic acids consists essentially of single-stranded antisense strand-nucleic acid fragments; and, elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set
15 of nucleic acid fragments.

159. A method of recombining a set of nucleic acid fragments, the method comprising:

20 hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded antisense strand-nucleic acid templates and a second set of nucleic acids consists essentially of single-stranded sense strand-nucleic acid fragments; and, elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set
25 of nucleic acid fragments.

160. A method of recombining a set of nucleic acid fragments, the method comprising:

hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded nucleic acid templates and a second set of nucleic acids comprises at least one set of nucleic acid fragments; and,

5 elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments by incubating the hybridized nucleic acid fragments with a polymerase and/or a ligase at a temperature of about 45°C or less, to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates,
thereby recombining the set of nucleic acid fragments.

10 **161.** The method of claim 113, wherein the hybridized nucleic acid fragments are incubated with a polymerase and/or a ligase at a temperature of about 37°C or less.

15 **162.** The method of claim 113, wherein the hybridized nucleic acid fragments are incubated with a polymerase and/or a ligase at a temperature of about 25°C or less.

163. A method of recombining a set of nucleic acid fragments, the method comprising:

providing a set of at least partially double-stranded nucleic acids that encode a polypeptide of interest or portion thereof;

20 contacting the set of at least partially double-stranded nucleic acids with an exonuclease that selectively degrades one strand of the at least partially double-stranded nucleic acids to provide a set of single-stranded nucleic acid templates;

hybridizing the set of single-stranded nucleic acid templates with a second set of nucleic acids comprising at least one set of nucleic acid fragments; and,

25 elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates, thereby recombining the set of nucleic acid fragments.

164. The method of claim 116, wherein the exonuclease is selected from the group consisting of Exonuclease III, Ba131, Mung bean nuclease, T7 gene 6 exonuclease, and lambda exonuclease.

5 165. The method of claim 116, wherein the nucleic acid fragments are single stranded.

166. A method of recombining a set of nucleic acid fragments, the method comprising:

10 hybridizing at least two sets of nucleic acids, wherein a first set of nucleic acids comprises single-stranded nucleic acid templates and a second set of nucleic acids comprises at least one set of nucleic acid fragments;

elongating, ligating, or both, sequence gaps between the hybridized nucleic acid fragments to generate at least substantially full-length chimeric nucleic acid sequences that correspond to the single-stranded nucleic acid templates;

15 introducing one or more of the at least substantially full-length chimeric nucleic acid sequences into at least one cell;

expressing the one or more introduced at least substantially full-length chimeric nucleic acid sequences to provide at least one expression product to the at least one cell; and,

20 selecting or screening the at least one cell for one or more desired traits or properties using at least one plate-based or at least one filter-based assay.

Add #1